

Editorial

A New Year in plant science

The New Year is an interesting watershed in any aspect of life, offering the opportunity to revisit the achievements of the previous year and to consider plans for the year ahead. For *New Phytologist*, 2007 was our busiest year ever in terms of manuscript submissions, with a 20% increase being recorded compared with the previous year. However, our service to the authors remains undiminished, with an average time-period of only 28 d from receipt of a manuscript to the first decision from the Editor. The combination of speed, efficiency and accuracy in dealing with submissions is the result of a large team, all of whom are thanked profusely. The team consists of an Editorial Board of 21 Editors, the Central Office with five staff, 69 Advisors to the board and more than 1200 Referees who reviewed manuscripts in 2007 – a total of 1295 devoted to publishing quality plant science research.

What might come the way of *New Phytologist* in this New Year? We have relied on the opinions of the Editorial Board for a glimpse of a future with new and emerging opportunities in plant science research. Although the interests and expertise of the board are very broad, there was a rather widespread view that very large data sets of biological information will increasingly underpin new synthetic and systems understanding. The most obvious area is in molecular plant science, where high-throughput technologies and reducing costs for DNA sequencing, for a wide range of plant and fungal species, provide the underpinning for insight into how plants work, from the cell to the whole organism. Genomic data are increasingly complemented by other 'omic' approaches, such as transcriptomics, proteomics and metabolomics. These data sets also create a problem in terms of accumulating, managing and interpreting them. However, they also provide completely new opportunities for understanding how interacting networks work and respond, not only to environmental challenges but in major processes that are poorly understood, such as developmental epigenetics and plant signalling.

The level of genetic and molecular detail varies considerably, with some model species, such as *Arabidopsis thaliana*, particularly well quantified. However, association genetic approaches offer a means for identification of the genes that cause adaptive

variation, even in virtually wild taxa such as *Pinus*. Although it is clear that the large majority of genes are common in their basic functions between widely different species, it may not be the case that the same phenotypic functions emerge.

Interactions between plants and other organisms, such as in mycorrhizal associations, but also with fungal endophytes and insects, are important but difficult to study; here again the data provided by mass-sequencing technologies will provide novel catalogues of processes occurring within and between organisms and in response to the environment. This rapidly growing field of ecosystem-level, multi-organism genomics has been termed 'metagenomics'.

Progressing from sequence collections to whole-system understanding uses new tools of bioinformatics, mathematical statistics and simulation modelling. This development is a feature of many aspects of plant science, not just genomics. In ecosystem studies the increased resolution and measurement of organismal activity, in both time and space, indicates a complex array of responses. Just like the equivalent in accumulating molecular sequences, this new proliferation of data results from new technologies, and just like the molecular area it still remains to be determined how this complexity of responses determines the response of the ecosystem, as a whole, to environmental changes. It is notable that new understanding in plant physiology, in particular the controls of photosynthetic carbon dioxide exchange, is now quite central to parallel fields of research such as hydrology, meteorology and atmospheric chemistry, yet this was very rare, even over a decade ago.

So we see another exciting year ahead with a whole suite of emerging opportunities providing a new raft of papers to further the development of plant science. We look forward to seeing them in *New Phytologist*.

F. Ian Woodward
Editor-in-Chief

Holly Slater
Managing Editor

Key words: DNA sequencing, epigenetics, high-throughput technologies, metagenomics, networks, photosynthetic carbon dioxide exchange, plant signalling, whole-system understanding.

Commentary

Remembrances of an embryo: long-term effects on phenology traits in spruce

Coping with their sessile life style, plants have become masters in adaptation. Through adaptation, they improve their chances of survival and reproduction in a potentially changing environment. Within the frame of optimal adaptation, phenology – the seasonal timing of growth processes – determines to a large extent the geographic distribution of many plants. By consequence, phenology traits often display some kind of latitudinal, altitudinal or drought clines that have been traditionally explored in breeding programs and provenance trials.

In Norway spruce (*Picea abies*), the translocation of northern ecotypes (64°–66°N) into a southern orchard (58°N), for better seed production, resulted in progeny that no longer resembled the phenology of their siblings produced *in situ* in the northern location. This effect persisted for years in the progeny (Skrøppa *et al.*, 2007). In line with this, the inverse experiment (translocation of a southern ecotype to the north) also produced, within one generation, progeny that were adapted to the northern location (Ø. Johnsen & T. Skrøppa, pers. comm.). Importantly, this observation is not limited to ecotypes that are displaced into a different environment, but extends to phenology characteristics of seedlings that were produced in warm or cold years within the same stand (Kohmann & Johnsen, 1994). In addition to Norway spruce, similar effects have only been demonstrated in *Picea glauca* × *Picea engelmannii* and in Scots pine (*Pinus sylvestris*; Dormling & Johnsen, 1992; Webber *et al.*, 2005). Quite surprising is the lack of substantial evidence on comparable phenomena in angiosperm trees, leaving the generality of the phenomenon an open issue.

‘... increased genome dynamics including epigenetic components might be a prerequisite for increasing the raw material for adaptive evolution.’

The molecular basis of these long-term effects in Norway spruce is currently unexplained. Johnsen and co-workers have established that the prevailing temperature during zygotic embryogenesis and seed maturation sets the phenology response and that the memory is probably of an epigenetic nature (Johnsen *et al.*, 2005a,b). Now, Johnsen and colleagues have repeated the original experiments with somatic, instead of zygotic, embryos and present their findings in this issue of *New Phytologist* (Kvaalen & Johnsen, pp. 49–59). The strength of this approach is provided by the elimination of genetic effects through the use of clonal material and a focus on temperature effects during zygotic and somatic embryogenesis. Kvaalen & Johnsen demonstrate that somatic embryos derived from identical genetic material, but exposed to different temperatures during zygotic and somatic embryo development, show marked differences in the critical night length for bud set in the second year of seedling growth. The difference in critical night length for bud set amounts to a remarkable 2 h, corresponding to 4–6° latitude in Norway. Furthermore, genetic selection during prezygotic stages, or selection of a particular embryo, could differ with varying temperature environments, but were excluded through demonstrating the absence of any genetic marker distortion (Besnard *et al.*, in press).

These temperature effects on the long-term phenology of the progeny do clearly differ from maternal provision or maternal effects *sensu stricto*. Maternal provision usually results in better-nurtured seeds that in turn have an obvious advantage during seedling establishment. The somatic embryos germinate at the same frequency, regardless of whether they have derived from a cold or a warm environment during embryogenesis and maturation, refuting an after-effect as a result of different maturity of the embryos or maternal provision. Moreover, the somatic embryos develop detached from the mother, identifying the embryo as the entity that senses and responds to temperature. In the provenance trials it is thus possible that zygotic embryos, rather than the mother tree, sense temperature. However, the independence of the memory effect from the mother plant or the female gametophyte remains to be established for zygotic embryos. Still, the maternal environment might influence the embryo sensitivity and response to temperature, or affect the magnitude of the epigenetic memory. Thus, the temperature-based memory effects in Norway spruce may not conform to all tenets of a typical maternal effect, still, the mechanisms underlying maternal effects can be useful for understanding the putative basis of the memory effects.

Maternal effects as a mechanism for adaptive transgenerational phenotypic plasticity

Maternal effects as a mechanism to pass an acquired adaptation to progeny are usually observed if the progeny are likely to live in the same environment as the mother plant (Galloway, 2005). In Norway spruce, the temperature during seed development depends on latitude, altitude and microclimate, and might thus correlate closely with day length and temperature that will govern the bud set of future progeny living in the same habitat. Maternal effects are particularly important if the spatial scale of environmental variation is greater than that of pollen movement or seed dispersal (Galloway, 2005), as is the case for Norway spruce. If habitat patches (micro-environments) display continuity over generations, then maternal effects will lead to phenotypic adaptation to the environment, thus, to habitat selection.

The effects of maternal environment have been documented in ecological studies, although the gain in fitness is often not described (Galloway, 2005). In yellow monkeyflower (*Mimulus guttatus*), for example, simulated insect damage on early leaves provokes increased trichome density on later leaves (within the generation) and in yet-unchallenged progeny originating from treated parents (across generation; Holeski, 2007). In American bellflower (*Campanula americana*), the offspring life style (annual vs biennial) is influenced by the maternal light environment in its forest habitat. This transgenerational plasticity is adaptive when offspring are grown in their maternal light environment (understory vs light gap), where seeds typically disperse. Offspring developing in light gaps that coincide with the environment of the mother show a 3.4-fold increase in fitness over those grown in understory (Galloway & Etterson, 2007).

Maternal effects can thus constitute a source of adaptive plasticity between generations, in which the offspring are predisposed with enhanced fitness to the environment they are likely to experience. The mechanism by which the maternal environment experienced during seed development imposes a particular disposition to the offspring is not yet understood. Moreover, it is unknown for how long these effects exert constraints upon the progeny.

Epigenetics as a basis for memory within and across generations

DNA methylation, chromatin and noncoding RNAs that, in turn, govern changes in DNA methylation and chromatin, are the molecular basis for epigenetic effects (Henderson & Jacobsen, 2007). The involvement of epigenetic components in the regulation of gene expression has clearly come to our attention. Environmental factors can play a substantial role in modulating the different epigenetic systems, as evident from the vernalization response in *Arabidopsis*. A prolonged period, but not short episodes, of cold lead to covalent histone modifications

of particular loci that are involved in the stable repression of flowering time locus C (*FLC*), a repressor of flowering in *Arabidopsis* (Sung & Amasino, 2005). A similar mechanism may explain the memory effect in spruce, although vernalization is by comparison a short-term memory.

Perhaps less recognized is that various environmental stresses influence genome stability, as documented for the activation of transposons in maize, genomic rearrangements in flax and altered frequencies of homologous recombination (Bond & Finnegan, 2007). In one very instructive example from *Arabidopsis*, Molinier *et al.* (2006) applied ultraviolet light and a bacterial peptide mimicking pathogen infection to young plants. Both stresses provoked the parental generation and four (unchallenged) filial generations to have higher frequencies of homologous recombination. The adaptive value of higher recombination frequencies with respect to the applied stresses is unexplained. Molinier *et al.* (2006) propose an epigenetic mechanism that possibly acts through alterations in chromatin condensation to make loci more accessible for recombination. Another study of a methylation-deficient *Arabidopsis* mutant showed that methylated CpG are central to epigenetic memory across generations (Mathieu *et al.*, 2007). Loss of methylated CpG triggered other epigenetic mechanisms, but these acted in an uncoordinated manner. Together, both studies established an epigenetic mechanism for transgenerational memory and underscore a central role for DNA methylation for its stability. Similar mechanisms, whether requiring a transgenerational component or not, might apply for the observed memory effects in Norway spruce.

The 'problematic' aspect of epigenetic mechanisms is their variable inheritance. They are frequently heritable through mitosis, sometimes for multiple generations (Richards, 2006). Thus, during sexual reproduction epigenetic information is partly reset during meiosis and partly transmitted through meiosis. The epigenetic marks at the *FLC* locus involved in the vernalization response are reset during meiosis, whereas transposon methylation is stably maintained (Bond & Finnegan, 2007). Nevertheless, increased genome dynamics, including epigenetic components, might be a prerequisite for increasing the raw material for adaptive evolution (Kalisz & Purugganan, 2004; Rapp & Wendel, 2005). Rapp & Wendel (2005) suggest that a population bottleneck, while reducing genetic diversity, might create at the same time epigenetic novelty. In contrast to genetic alleles, epialleles might react more quickly to environmental change, be reversible and persist for a number of generations only (Kalisz & Purugganan, 2004). If the epiallele were to cause a mild phenotype, through an alteration of the degree of gene expression, it might experience weaker selection than a loss-of-function sequence mutation (Kalisz & Purugganan, 2004). The significance of epialleles in wild populations will depend on their frequency and stability.

In conclusion, initial reports establish epigenetic effects as a basis for memory within and across generations. Future studies are needed to investigate whether and to what extent adaptation

through phenotypic plasticity, such as reported by Kvaalen & Johnsen, relies on epigenetic mechanisms.

Implications of an epigenetic memory for bud set

Bud set is among the most differentiated genetic traits in many temperate trees (Howe *et al.*, 2003). Along latitudinal and altitudinal clines strong genetic differentiation prevails for phenology traits, typically resulting in locally adapted ecotypes. The memory mechanism suggested by Johnsen and co-workers would counteract this strong differentiation through introducing new sources of phenotypic variation at the local community level, provided it were a widespread phenomenon in the wild and that successive seed years varied considerably in temperature.

The strong differentiation or genetic constraint, on the one hand, and counteracting mechanisms that introduce variation and/or phenotypic plasticity from various sources, on the other, might of course reflect flip sides of the same trait. Alternatively, the clinal gradient in bud set might mask a very large within-stand variation. Conventional provenance-level quantitative genetic variation (i.e. that among geographic origins) might reflect both the directional selection and the phenotypic plasticity. The ease of provenance transfer in Norway spruce is interpreted as a sign of phenotypic plasticity (Skrøppa *et al.*, 2007). To achieve reliable estimates of phenotypic plasticity (magnitude of effect and its heritability), clonally replicated progeny of a cross would need to be assessed over a variety of environments (Pigliucci, 2005). Additionally, no quantitative methods currently exist to distinguish between genetic and epigenetic components affecting bud set. Whether contributing to trait variance (covered in the genetic variance) or to variation in developmental stability (contained in phenotypic variance), epigenetic components are currently undetected (Kalisz & Purugganan, 2004). Epigenetic effects might thus inflate the variation in bud set among the provenances, causing the clines to reflect more local adaptation than actual genetic differences can account for. Taken to the unlikely extreme, if provenances indeed adapt within one generation to the local conditions, much of the phenotypic plasticity might come through embryo memory and/or maternal effects.

There are uncertainties as to whether this long-term effect on phenology will lead to a comparable outcome and significance in wild populations that will experience warmer temperatures as a result of global warming. The discussion continues as to whether evolutionary adaptation could cope at all with the expected speed of climate change (Huntley, 2007; St Clair & Howe, 2007). The contribution of adaptive genetic evolution and phenotypic plasticity to the already observed phenological changes in the past decades is currently unknown. However, within a 100-yr timeframe for strong climate change, migration and evolution in place are rather unlikely scenarios for adequate genetic adaptation, particularly for forest trees. Eurasian Scots pine (*P. sylvestris*) was predicted to need as many as

12 generations to evolve to the new optima in future climates (Rehfeldt *et al.*, 2002). Discussing this apparent dilemma for Douglas fir (*Pseudotsuga menziesii*), St Clair & Howe (2007) recommend human interference through movement of populations from south to north, from lower to higher altitude and the deployment of mixed-seed sources to ensure forests that can face future climates. However, perhaps the within-population variation in climatic responses is greater than often assumed, even in marginal areas (Johnsen & Østreg, 1994). Within this scenario, the observed memory effect might constitute a source of phenotypic plasticity, although the actual contribution of this memory effect and the putative maternal influences to the total phenotypic variation in natural populations remain to be uncovered.

Research needs

Future experiments are needed to assess whether it is also the case for zygotic embryos that epigenetic memory is established within the embryo itself and whether or not it involves transgenerational inheritance from the parental generation. Reciprocal hybrids can unequivocally establish to what extent gene expression is affected by maternal effects (affecting both paternal and maternal alleles *in trans*) or by genomic imprinting (specific to only one parental allele). Furthermore, systematic analyses of the epigenome should ascertain the epigenetic nature of the phenomenon. A first estimate of the extent of methylation can be provided using methylation-sensitive amplified fragment length polymorphism (AFLP) markers. Other viable approaches include the study of specific genes involved in phenology responses, such as the three phytochrome genes whose expression levels in seedlings appeared to correlate with different temperatures during zygotic embryogenesis (Johnsen *et al.*, 2005a). The methylation patterns of these and other genes should be determined in both parental gametes, in the zygote and in the seedlings to demonstrate whether methylation patterns are established *de novo*.

In addition to bud set, the warmer temperature during embryogenesis and seed maturation leads also to delayed bud burst and dehardening in spring, to later bud set, to delayed frost-hardiness development and to delayed lignification (Johnsen *et al.*, 2005a,b). Although all traits could depend on one pleiotropic regulator, it is also possible that the higher temperature during seed development results in a nonspecific alteration of chromatin configuration that would affect many traits. Monitoring constructs, such as those used for quantifying homologous recombination in *Arabidopsis* (Molinier *et al.*, 2006), could reveal such alterations. Similarly, general loss-of-function approaches to perturb or erase the memory should clarify whether DNA methylation or chromatin configuration were the molecular basis of the memory.

Much of the suggested experimentation is highly speculative and would be extremely challenging, if not impossible, in

Norway spruce – a species in which intensive molecular studies are very difficult. This situation calls for a main research need in this area, namely whether comparable effects could be identified in model plants that are more amenable to molecular methods. Potentially, the lack of data in other (tree) species is the result of mere ignorance of these phenomena, as there appear to have been very few, if any, serious attempts at study. Conifers are, however, distinct in having very large genomes that possess a higher abundance of epigenetic mechanisms in place to control the repetitive parts of the genome. It is therefore feasible that in conifers such as Norway spruce, this epigenetic regulation might be more easily recruited for the regulation of other processes. Nevertheless, whatever the reasons underlying the observations of Kvaalen & Johnson, they reveal an important mechanism for adaptation to new and unstable environments. Knowledge on adaptation is so fundamental for forestry and forest genetic conservation that these memory effects deserve further investigation.

Antje Rohde^{1*} and Olavi Junttila²

¹Institute for Agricultural and Fisheries Research,
9090 Melle, Belgium;

²Institute of Biology, University of Tromsø,
9037 Tromsø, Norway

(*Author for correspondence:
tel +32 (0)9 272 2953; fax +32 (0)9 272 2901;
email antje.rohde@ilvo.vlaanderen.be)

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Key words: adaptation, epigenetic memory, maternal effect, Norway spruce, phenology, phenotypic plasticity.

C₄ leaf curling – coupling incident light, stomatal and photosynthetic asymmetries

Patterns in leaf structural asymmetry among vascular plants have prompted a long-standing and continuing interest in the effects on photosynthetic performance (DeLucia *et al.*, 1991). Leaf curling, most often interpreted as a response to water stress (e.g. Heckathorn & DeLucia, 1991), represents a dynamic behavioral response in plants whereby the normal sunlight orientation of the two leaf surfaces may be reversed.

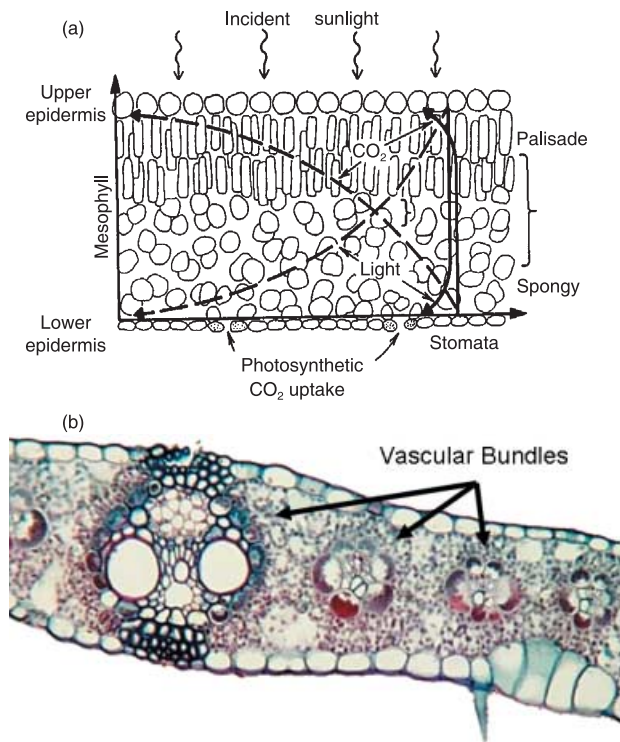


Fig. 1 Typical C_3 and C_4 leaf structure. (a) The well-documented differentiation of mesophyll cells (e.g. palisade and spongy) in the thicker sun leaves of C_3 species enhances propagation and dispersal of absorbed sunlight (shown schematically), while an increasingly equal distribution of stomata on the two leaf sides significantly enhances CO_2 supply. The hatched curve represents no structural effects on internal gradients; solid lines show the increased overlap of absorbed light and CO_2 resulting from structural effects. (b) The typical leaf structure of C_4 species (e.g. grasses and other monocots) includes a more equal distribution of stomata on the two leaf sides, but no strong differentiation of cells (concentrated around the vascular tissue bundles, i.e. 'Kranz' anatomy) that might enhance light and CO_2 overlap inside the leaf.

The large majority of plant species (those with the C_3 metabolic pathway) have leaves that are oriented approximately horizontally and have an accompanying asymmetry (dorso-ventral) in both external morphology (e.g. stomatal distribution) and internal anatomy (e.g. differentiated mesophyll cells) (Fig. 1a). This leaf asymmetry generates the well-known sun vs shade leaf structure that can function to increase the overlap of absorbed sunlight and CO_2 inside the leaf, and thus photosynthetic efficiency (Smith *et al.*, 1997). In contrast, there are also numerous plant species that have a more inclined leaf orientation and do not have sun/shade differences in leaf structure, but have more equal numbers of stomata on the two leaf surfaces. In particular, species with C_4 metabolism often have a distinct 'Kranz' internal anatomy (chlorophyll-containing cells concentrated around vascular bundles) that is perceived as not having the structural capability to regulate the distribution of absorbed sunlight inside the leaf (Fig. 1b). Rather, CO_2 -concentrating mechanisms

and increased phloem loading are known to contribute to high photosynthesis in C_4 species, particularly in high-sunlight environments. In this issue of *New Phytologist*, Soares *et al.* (pp. 186–198) report on an imaginative and thorough set of experiments showing that a C_4 grass species, during natural leaf curling that reversed the orientation of the upper and lower leaf surfaces, had accompanying stomatal and biochemical changes inside the leaf that enhanced its photosynthetic capability. Remarkably, stomata closed on the opposite, newly shaded side of the leaf, while adjacent cells appeared completely inactivated photosynthetically. This dramatic asymmetry in stomatal and photosynthetic function occurred only when the lower leaf surface became illuminated via curling. Thus, the photosynthetic response to sunlight incidence on a particular leaf side was not based on leaf structural differences determined during leaf development, as commonly reported in C_3 species. Instead, a more dynamic, biochemical response occurred that linked sunlight incidence to the behavior of stomata and photosynthetic cells throughout the full thickness of the leaf. This rapid response capability during leaf curling suggests an alternative adaptive venue in C_4 plants, one that is much more temporally dynamic than the developmentally determined changes in leaf structure found in C_3 plants (i.e. sun/shade leaves). Photosynthetic enzymes (e.g. Rubisco and phosphoenolpyruvate carboxylase (PEPC)) were also activated in photosynthetic cells near the sunlit surface with open stomata, where both light intensity and CO_2 concentration were high. Although not reported in Soares *et al.*, a major enhancement in leaf water use efficiency (during leaf curling) would be expected.

'This dramatic asymmetry in stomatal and photosynthetic function occurred only when the lower leaf surface became illuminated via curling'

Photosynthetic asymmetry in plant leaves

Despite the major differences in structural leaf symmetry found within the plant kingdom, relatively little information exists on specific photosynthetic impacts of sunlight incidence on different surfaces of the same leaf. In fact, few measurements exist that quantify differences in sunlight incidence on each leaf side under natural field conditions. During typical photosynthesis measurements in the field or laboratory, only the amount of downward, hemispherical irradiance is typically measured, without concern for the amount of sunlight striking both leaf surfaces. Yet, a host of leaf structural parameters with

documented photosynthetic impacts have been strongly associated with both the amount of incident sunlight on each leaf surface and the ratio of these amounts (Smith *et al.*, 1998). Also, the stomata of several understory species, found predominately on the lower leaf surface that received low and slowly changing sunlight incidence, responded linearly to sunlight incidence on the upper-facing leaf surface during morning stomatal opening (Smith, 1981). The mechanistic process by which plant leaves sense and respond photosynthetically to asymmetric sunlight incidence is unknown; in particular, the coordination of the stomatal uptake (CO_2 supply) and the CO_2 demand of the sunlit photosynthetic cells within the leaf remains to be elucidated.

Ideally, for plant leaves, there should exist a CO_2 supply and demand control system that is coupled tightly to sunlight incidence. In C_3 species, characteristic changes in leaf structure as a result of changes in irradiance intensity occur only during developmental cellular differentiation (e.g. Smith *et al.*, 1997). Even the unusual leaf structure of the needle-like leaves of conifer species appears to utilize another, purely structural adaptive strategy (more cylindrical leaf morphology and radial anatomy) that replaces the functional benefits of typical C_3 cell differentiation to photosynthetic performance (Johnson *et al.*, 2005).

Experimental approach

A clever and insightful aspect of the approach of Soares *et al.* was the selection of a C_4 species with nearly equal numbers of stomata on the two leaf sides, and a characteristic leaf structure that is, typically, highly symmetric compared with C_3 plants. The choice of a C_4 species eliminated the possibility of an effect of leaf structure on sunlight distribution and CO_2 diffusion inside the leaf, an effect that is already known to exist for C_3 species. Also, the fact that this class of plants can assimilate internally generated CO_2 (from respiration and photorespiration) for photosynthesis in the absence of light, and with closed stomata, was an important part of this experimental design. Thus, differences in the response of stomata to incident sunlight (adaxial or abaxial) were measured experimentally, and additional, supporting measurements were made that would add credibility to their conclusions about mechanisms (see 'Supporting data' below). The investigators' design of a CO_2 gas exchange system that enables accurate measurement of photosynthetic CO_2 exchange from the whole leaf, as well as from each of the two leaf surfaces separately, is also a significant contribution to the field.

Supporting data

Soares *et al.* also provide data showing the effects of elevated ambient CO_2 (during growth) and the more instantaneous interactions with the calculated concentration of CO_2 in the

intercellular air spaces (C_i) between the stomata and the photosynthetic cells. This C_i term is easily calculated from standard gas exchange measurements and is often employed to help understand the dynamics involved in the coordination of stomatal supply of CO_2 vs the demand by the photosynthetic cells inside the leaf. For example, a high C_i may indicate either a low cellular demand (often stress related), or a degree of stomatal opening that is unnecessarily high and, thus, expensive in terms of transpirational water loss. A low C_i value might reflect a strong stomatal limitation where CO_2 supply is not keeping up with CO_2 demand.

In summary, the following supportive data presented by Soares *et al.* eliminated other possible mechanisms that might provide alternative explanations for their conclusions.

- Photosynthesis was equal on the two leaf sides when illumination was on the adaxial side, but was asymmetric and substantially greater overall when the abaxial side was illuminated, with zero photosynthesis and complete stomatal closure occurring adaxially.
 - When light was directed to the adaxial surface, stomata closed on both leaf surfaces, even under decreasing C_i conditions when stomatal opening should typically increase.
 - Illumination of the abaxial surface (simulating leaf curling effects) led to complete stomatal closure on the adaxial surface and complete cessation of leaf photosynthesis, indicating no internal transport of CO_2 from the leaf side with open stomata (i.e. abaxial) or photosynthetic assimilation of metabolically generated CO_2 on the side with closed stomata and low illumination.
 - No differences in whole-leaf optical properties (absorptance, reflectance, and transmittance) occurred for one leaf side vs the other.
 - Photosynthetic enzyme proteins were distributed uniformly across the leaf thickness in plants grown under unenriched CO_2 .
- Growth under enriched CO_2 increased twofold, but did not appear to influence substantially the dorso-ventral regulation of photosynthesis based on light incidence on a particular leaf surface. However, enrichment and abaxial illumination did result in a slight decrease in the response of photosynthesis to changes in C_i .

Concluding perspectives and future research

The above findings suggest a regulatory signaling by light-activated stomata that is transmitted across the full thickness of the leaf and causes stomatal closure on the opposite, now low-light leaf surface. This sort of tight coupling between cells experiencing different environmental conditions (sunlight and CO_2 concentration) has not been reported before. The rapid transfer of environmental signals to a physiological process over the entire leaf thickness may be similar to the sensing and transfer of environmental signals (e.g. ambient CO_2 concentrations) over even greater distances; for example, a signal to alter stomatal size and frequency from a mature leaf

to a developing leaf still in the bud stage (e.g. Lake *et al.*, 2001). Such a rapid intercellular communication linking the environment with leaf photosynthesis is an important area for future research (Hanstein & Fell, 2004; Thomas *et al.*, 2004; Yano & Terashima, 2004).

One interesting report involving the interaction of C_i with the possible control system proposed by Soares *et al.* is the spatial segregation of an intercellular air space that is connected separately to each leaf surface (Long *et al.*, 1989). These results also have functional significance for both the lower (e.g. chloroplast location) and higher (e.g. ecosystem) organizational scales defining the importance of C_4 species. These species are photosynthetically distinct in their CO_2 -concentrating physiology and accompanying high productivity, and are currently targeted for agricultural/commercial development into bio-energy products (e.g. bio-fuel). The authors also point out that the future performance of C_4 species under current scenarios of global change (e.g. elevated CO_2), compared with the much more abundant C_3 species, has not received much attention.

William K. Smith

Department of Biology, Wake Forest University, Box 7325,
Winston-Salem, NC 27109, USA (tel +1 336 7585779;
fax +1 336 7586008;
email smithwk@wfu.edu)

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Key words: leaf curling, photosynthetic asymmetry, stomata, sunlight incidence.

Plant disease and global change – the importance of long-term data sets

Research on the interactions between climate change and plant diseases is rapidly catching up with the importance of the topic (Garrett *et al.*, 2006). On the one hand, modelling studies are providing increasingly realistic scenarios for the influence on plant diseases of changes in the magnitude and variability of temperature, precipitation and other climatic variables. Recent examples include models predicting an increase under projected climate change in (i) severity of *Plasmopara viticola* epidemics on grapes in an important wine-producing Italian region near Turin in 2030, 2050 and 2080 (Salinari *et al.*, 2006), (ii) the range and severity of epidemics of *Leptosphaeria maculans* on oil seed rape (*Brassica napus*) in the UK for the 2020s and 2050s (Evans *et al.*, in press), and (iii) the distribution and local impact of a range of forest pathogens (*Biscogniauxia mediterranea*, *Cryphonectria parasitica*, *Melampsora* spp., *Phytophthora cinnamomi* and *Sphaeropsis sapinea*) in France at the end of the 21st century (Desprez-Loustau *et al.*, 2007a). On the other hand, short-term, local experiments have demonstrated the impacts of predicted global change on plant health. Recent examples include: (i) a study showing that elevated atmospheric CO_2 concentration increases the risk of infection with rice blast (*Magnaporthe oryzae*) and the percentage of rice (*Oryza sativa*) plants affected by sheath blight (Kobayashi *et al.*, 2006); (ii) an experiment demonstrating species-specific responses to increased ozone concentrations of the susceptibility of young beech (*Fagus sylvatica*) and spruce (*Picea abies*) trees to *Phytophthora citricola* (Lüdemann *et al.*, 2005); and (iii) a 12-yr warming experiment with heaters suspended over plots in a mountain meadow in Colorado, USA in which there was a change in the prevalence of different species of plant pathogens (Roy *et al.*, 2004). These approaches are complementary, as they each have limitations. Modelling can only work if the long-term predictions are not overturned, for example by new insights into the co-evolutionary dynamics of plant–pathogen interactions; and short- to medium-term local experiments may not take into account time lags and signal accumulations (for nitrogen (N) fertilization: Strengbom *et al.*, 2001; for CO_2 concentration: Körner, 2006; for N deposition and

raised temperature: Wiedermann *et al.*, 2007). A study by Shaw *et al.* in this issue of *New Phytologist* (pp. 229–238) provides an example of how the limitations of models and experiments can be overcome by making use of a long-term (1844–2003) UK data set on the occurrence of two key world-wide pathogens of wheat (*Triticum aestivum*) (Stukenbrock *et al.*, 2006).

'The use of herbarium specimens in this way and their analysis using PCR techniques provide a unique way of characterizing changes in pathogen prevalence over historical time.'

In the Rothamsted Broadbalk experiments, a series of wheat plots each receiving a different amount of fertilizer have been grown and monitored almost continuously since 1843. Using PCR data from those plots, Shaw *et al.* combine the rigour of an experimental setting with the advantages of over a century of data. They show that in the long run the annual incidences of *Phaeosphaeria nodorum* and *Mycosphaerella graminicola* in the grain of wheat are, respectively, positively and negatively related to the emission of sulphur dioxide (SO₂) across the whole of the UK and that these associations largely account for the variations in the relative prevalences of the two pathogens over this period of time. The use of herbarium specimens in this way and their analysis using PCR techniques provide a unique way of characterizing changes in pathogen prevalence over historical time. Emissions and atmospheric concentrations of SO₂ have markedly declined over the last two decades in Europe and North America, thus roughly going back to the concentrations observed at the start of the Rothamsted experiment (*c.* 1–2 megatonnes of sulphur per year; Bearchell *et al.*, 2005). The decline in SO₂ emissions in Europe has also been related to improvements in forest health (e.g. Zirlwagen *et al.*, 2007), and it would be interesting to know whether forest tree endophytes (which can behave as mutualists or not, depending on host conditions; Sieber, 2007) have responded in a similar way to wheat pathogens. SO₂ emissions have recently increased in China, where forest health is generally declining (e.g. Wang *et al.*, 2007). The findings of Shaw *et al.* suggest that the relative prevalences of *P. nodorum* and *M. graminicola* in China's wheat fields should have gone the opposite way to those at Rothamsted. However, Shaw *et al.* also provide evidence that the short-term variability in the presence of these two pathogens in the leaves of wheat is associated with

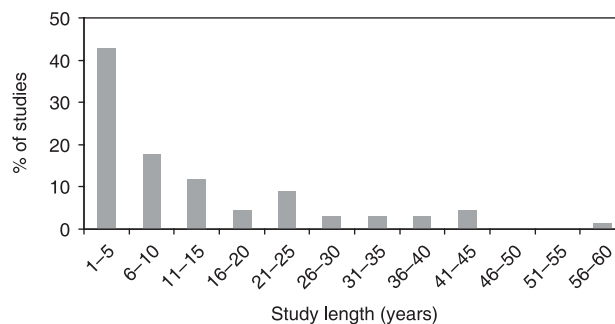


Fig. 1 Frequency distribution of 67 studies modelling tree mortality published between 1997 and 2006, according to the length of the period upon which models were based. Sixty-seven of the 141 papers retrieved contained data about the study plots used to validate models. About 60% of these 67 models were based on surveys of less than 10 yr.

weather conditions such as summer temperature and spring rainfall. Together with sea levels (DeSantis *et al.*, 2007), temperature and rainfall are the two climatic factors that are most likely to be widely affected by future global change, and alterations in these factors are expected to have a wide range of impacts both on plants and on their pathogens (Ingram, 1999).

There is increased use in plant ecology of data sets extending over periods longer than one century, not only from fossil series, pollen records and dendrochronology (see also Woodward, 2007). For example, Tait *et al.* (2005) analysed changes in species richness and composition of the flora in the Adelaide Metropolitan Area for the period 1836–2002. For Turin, Italy, Isocrono *et al.* (2007) showed that lichen species richness has increased from 1792 to the present day, an indication of the improving air quality of that conurbation. For the flora of the Coliseum in Rome, Caneva *et al.* (2005) found a marked decrease in the presence of species typical of a cool and wet climate from 1643 onwards. For the living collections of the botanical gardens of the world, Pautasso & Parmentier (2007) showed that age (up to more than 400 years for the oldest gardens) explains one-fifth of the variance in the current species richness. Similar long-term data sets, however, are still rare, particularly in relation to plant and tree mortality (Fig. 1). In a recent example, an analysis of the genomes of barley yellow dwarf viruses in herbarium specimens (1894–1958) showed that this disease may have facilitated the invasion of introduced grasses in California (Malmstrom *et al.*, 2007). For the UK, Jones & Baker (2007) showed that, at least for the period 1970 to 2004, the number of recorded introduced plant pathogens has not increased with time. However, this might well change in a markedly different climate. The issue of exotic pathogens is of utmost importance in relation to climate change. Global warming will not only act on pathosystems already present in a certain region, but will favour the emergence of new diseases, both because the distributional range, temporal

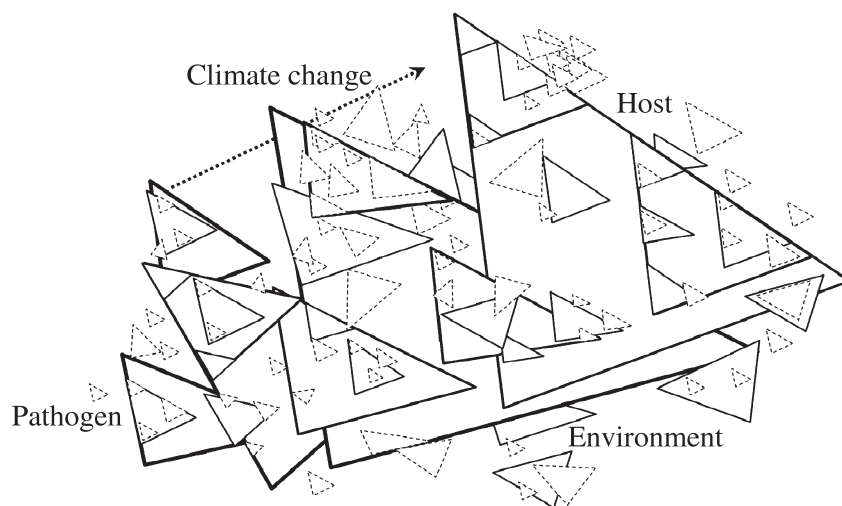


Fig. 2 A scale-dependent view of the effects of climate change on the disease triangle (this is formed by the interactions amongst host, pathogen and environment; see e.g. Scholthof, 2007). Climate change implies transformations of the environment of pathosystems over local, regional and continental scales, and these are in turn expected to increase the distribution and virulence of many plant pathogens, as well as cause a mismatch between host distributions and the range of conditions to which they are best adapted.

activity and community structure of pathogens will be modified (e.g. Desprez-Loustau *et al.*, 2007b; Shaw *et al.* 2007), and because the phenology and conditions of the hosts will be altered (e.g. Lonsdale & Gibbs, 1996). Add to this the long-distance introductions of pathogens as a result of the increasing globalization of trade (e.g. Jeger *et al.*, 2007) and the challenges for end-of-the-century plant pathology are likely to become as complex as they will be unprecedented (Fig. 2).

Not all changes in pathosystems are necessarily related to climate change (e.g. Rogers & Randolph, 2006), but the evidence that climate change can profoundly influence host–pathogen dynamics is growing, not only for plant diseases but also for animal and human diseases (e.g. Purse *et al.*, 2005; Haines *et al.*, 2006). There is a need not only for interdisciplinary collaboration between epidemiologists and climate scientists (Huntingford *et al.*, 2007), but also for more awareness of investigations relating to climate change and diseases of plants, animals and humans in the three scientific communities, as the science involved is similar and analytical techniques are transferable and do not need to be reinvented (see e.g. Cazelles *et al.*, 2007). One issue that is still rarely addressed and is not resolved by the availability of long-term data sets is the potential spatial scale dependence of responses of plant pathosystems to climate change, particularly if local studies focus on habitat patches where disease is disproportionately present (Holdenrieder *et al.*, 2004; Strengbom *et al.*, 2006). Climate change will affect plant pathosystems at a variety of levels of integration (from genes to populations and from ecosystems to distributional ranges) and in most aspects of epidemic development (from environmental conditions to host vigour and susceptibility, and from pathogen virulence to infection rates). Climate change is likely to have a profound impact on plant–pathogen interactions, and will thus represent a world-wide interdisciplinary challenge not only for the long-term sustainability of crop production but also for the understanding of biodiversity dynamics in a changing world and for the success of conservation biology activities.

Mike J. Jeger* and Marco Pautasso

Division of Biology, Imperial College London,
Silwood Park Campus, Ascot SL5 7PY, UK

(*Author for correspondence: tel +44 020 759 42428;
fax +44 020 759 42601; email m. jeger@imperial.ac.uk)

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Key words: disease triangle, forest health, global warming, introduced organisms, landscape pathology, plant pathosystems, SO₂ emissions, temporal scale.

Meetings

Systems biology and the biology of systems: how, if at all, are they related?

17th New Phytologist Symposium, Buxton, UK, September 2007

It is said that Einstein never liked the term 'relativity', and indeed the word had already been used previously by Poincaré in

connection with a rather different area of mathematical physics. Nevertheless, the expression quickly acquired a life of its own and soon 'relativity', 'relativistic' and so on became highly specific labels with tightly circumscribed connotations in the context of physics. The meanings of individual words matter, particularly when they can open up or narrow down whole areas of science.

We seem to be at such a point now with the term 'system' as used in biology. The founder of the present journal, Sir Arthur Tansley, introduced the concept of the ecosystem in 1935 (Willis, 1997). Agricultural systems have been recognized

and studied since science was first applied to food production. Most biologists, particularly those working at the physiological or ecological level, think they have always aimed for system-level understanding. What is new about systems biology (SB) as currently conceived? It seeks to bring together understanding of structure (in terms of gene and biochemical networks), system dynamics (involving predictive modelling), system control methods and system design. But crop scientists, ecologists, developmental biologists and the like who use tools such as 'omics, large-scale data capture, informatics and modelling will say that they are also concerned with such systems properties.

Hence the title of the symposium, which sought to clarify whether SB really is a new and exclusive form of biology, and how it might benefit, and benefit from the experiences of, biologists who already approach their subject in a system-orientated fashion. This concern was expressed bluntly, echoing the title of John Sheehy's (IRRI, Manila, Philippines) presentation, in the final discussion session led by Malcolm Bennett (University of Nottingham, UK): are terms such as 'plant systems biology' and 'crop systems biology' useful or useless?

'As one moves up the scale of biological complexity, the environment becomes a bigger, and increasingly uncontrollable, factor.'

What is a (biological) system and what are the benefits of analysing it?

Several speakers at the symposium set out what a biological system meant to them. Sheehy defined a system as 'a number of interacting elements existing within a boundary which is surrounded by an environment.' Interaction within a boundary is a common property of ideas about SB and the biology of systems. Interactivity takes the form of networks, circuits, feedback and feed-forward processes, iteration, recursion and proliferating complexity with increasing numbers of system components. Andrew Millar (University of Edinburgh, UK) visualized the SB discovery process as a cycle comprising data acquisition (typically in massive amounts, crossing time and length scales and dependent on powerful bioinformatics resources), analysis, modelling and validation and deployment of models for prediction and generation of new understanding. His own work on the circadian clock in *Arabidopsis* showed the SB approach in action. His group has developed a feedback loop-based model of gene interactions with the property of predicting

connections and additional components that could then be sought experimentally (Locke *et al.*, 2006). In this way they hypothesized the existence of a previously unsuspected gene (*Y*) and, by analysis of mutants, confirmed that *Y* is identical to the *GIGANTEA* gene. This work is beginning to link up with that on other species such as mouse and *Drosophila*, indicating the potential of the systems approach to establish broad biological principles.

June Medford (Colorado State University, USA) provided a remarkable glimpse of what might become possible when we gain functional understanding of the cellular circuitry of the plant (Antunes *et al.*, 2006). Exploiting recent advances in knowledge of the biochemistry and genetics of chlorophyll synthesis and degradation (Kr  tler & H  rtensteiner, 2006; Tanaka & Tanaka, 2006), her group has engineered plants that can report on specific abiotic stimuli in the environment by colour modulation. Noninvasive detection of pigmentation changes means that such plants become real-time sentinels. It is rare for participants at a plant science conference to witness images of detonations in a presentation, as happened here in discussions of the use of such sentinels to detect volatile explosives. The array of environmental sensor pathways that plants, as sedentary organisms, must deploy for acclimation, adaptation and survival makes them particularly suited for exploitation as sentinels, and SB can be expected to contribute knowledge for effective design of the underlying circuitry.

What types of data and tools are required for plant SB?

SB places great emphasis on massive data sets and the use of bioinformatics resources and computational power to shake meaning out of them. The implications of the data tsunami engulfing biology, the need for heavy-duty computing, data management and quantitative processing, and the issue of whether we are heading for a new kind of hypothesis-free science have been well aired and were discussed again at the present Symposium. Tools also need to be developed for quantifying attributes such as size and shape if models of growth and morphogenesis are to be brought into the same systems arena as the likes of 'omics. Andrew Bangham (University of East Anglia, Norwich, UK) described new approaches, illustrated by work on leaf growth and flower development. Time-lapse images of features tracked in two and three dimensions lead to models that can simulate closely the final forms of organs, how these were arrived at topologically and the morphogenetic fields that define them. These in turn are reflected in observed and hypothetical spatial patterns of gene expression (Lee *et al.*, 2006).

Just as observational approaches to system definition have undergone a major technology-driven quantitative and qualitative shift, so too is the world of plant modelling

developing new and powerful tools for representing structures, functions and morphogenesis. Gerhard Buck-Sorlin (Wageningen University, the Netherlands) described recent developments in functional-structural plant modelling (FSPM; Godin & Sinoquet, 2005), which aims to define the complex interactions between plant architecture and the physical and biological processes that drive plant development at several spatial and temporal scales (Buck-Sorlin *et al.*, 2005). Buck-Sorlin argued that FSPM is effectively the upscaled equivalent of SB. He presented a new synthesis of the traditional programming paradigms used in plant modelling (procedural, object-oriented, and rule-based) and showed how modularity and embedding one paradigm into another can extend the range of processes that can be modelled. The elegance of the approach was in this case matched by the aesthetics of the virtual roses presented as examples.

Jan Kim (University of East Anglia, Norwich, UK) described transsys (Kim, 2005), a computer language framework for modelling regulatory gene networks. The transsys framework supports construction of computer models integrating regulatory gene networks with other levels, such as morphogenesis, and provides optimization tools that fit parameters on a global, system level. This language may be integrated with the Lindenmayer (L-system; Prusinkiewicz & Rolland-Lagan, 2006) approach to morphological modelling. Kim, with Bangham, has shown how L-transsys can be used to model floral structures and their genetic variants.

Is SB the privilege of scientists working at the cell-to-molecular level?

Xinyou Yin (Wageningen University, the Netherlands) articulated the question that has been on most people's minds since SB arrived on the scene: are the rules of SB such that only the molecular and subcellular aspects of biology can be addressed through application of its approach? It is of concern to many plant scientists that the plant SB community will be more concerned with sustaining its credibility in the eyes of people in the yeast or T-lymphocyte business than with translating their advances into new understanding of higher-order plant phenomena such as photosynthesis, crop yield and ecological fitness. The answer, of course, is dialogue, together with demonstrations of convincing cases connecting gene circuitry with real phenotypes. Yin argued that a way forward is to use trait mapping linked at a reductionist level to molecular genetics and genomics, and at the extensive, phenotype level to crop process models (Yin *et al.*, 2004). He showed examples of the dissection of complex characters into component traits based on ecophysiological insight and indicated that the integrated approach gives better resolution of genotype-by-environment interactions. This view of crop systems biology is highly consistent with, and enriches, the long-established plant breeding concept of the ideotype (Donald, 1968).

Can SB approaches aid studies at higher physical scales?

The systems discussed at this meeting share the characteristics of hierarchical structure and emergent properties. Scaling is a profound challenge in biology. For example, there are conceptually and mechanistically indisputable and statistically rigorous models of photosynthesis that provide highly effective simulations of the biochemistry and physiology of the process (e.g. Yin *et al.*, 2006; Dubois *et al.*, 2007); but moving up to the whole-plant, crop or community level, photosynthesis as modelled in this way bears only the most limited predictive relationship to productivity and fitness. In fact we might as well use the term 'vanishing' rather than 'emergent' for the properties of systems at such higher scales.

The issue of emergent (or vanishing) properties was addressed in the presentation by Sheehy. He surveyed the general field of empirical and mechanistic models and focused on how modelling starts with observations at the whole-system level and progresses as understanding of the component parts and subsystems increases, resulting in a caricature of the system represented in equations. Much is made of SB as the means of brokering agreements among biologists, computer scientists, engineers and mathematicians to direct their efforts towards resolving common problems. In fact such a convergence of disciplines seems to happen, apparently spontaneously, once a generation or so. It happened in the middle decades of the last century when physicists and chemists made common cause with the geneticists to crack the structure of the gene. In more recent times, crop science in the run-up to the Green Revolution had attracted physical scientists (like Sheehy), statisticians and modellers to put breeding and agronomy on a quantitative basis (motivated in part by idealistic 'feed the world' sentiment). Sheehy showed how lessons learned from that turn of the cycle could and should inform how contemporary SB develops. He also argued that SB should contribute to meeting an urgent global objective – increasing crop yield. The strategy for addressing this through the creation of C₄ rice (Mitchell & Sheehy, 2006) provoked much discussion.

As one moves up the scale of biological complexity, the environment becomes a bigger, and increasingly uncontrollable, factor. This is an understandable justification for confining the practice of SB to the subcellular and the time-limited. However, important biological insights can be overlooked because close control of experimental conditions causes critical environmental interactions to be excluded. Two dramatic examples were provided by Stefan Jansson (Umeå Plant Science Centre, Sweden). He described work on the regulation of light harvesting in *Arabidopsis* (Külheim *et al.*, 2002) in which a predicted fitness disadvantage of knocking out a component of the feedback de-excitation process of photosynthesis could not be verified under standard controlled cultivation conditions. If, however, the knock-out population was grown in a natural environment, its performance was

significantly inferior to that of wild-type, measured in terms of a number of fitness parameters. In other words, the feedback de-excitation mechanism, which is pretty well described in molecular and biophysical detail and therefore clearly qualifies as a system in SB terms, did not reveal its true function and biological significance until it was analysed in an uncontrolled fluctuating and unpredictable environment. This is quite likely to be the rule for many of the critical adaptive and developmental systems of plants, and represents a challenge to some conventional SB thinking. Jansson also introduced a further example of the work of his group on 'real-world genomics'. Using DNA microarrays, they carried out transcript profiling in leaves of a single field-grown aspen tree (*Populus tremula*) over several years, focusing particularly on the period covering initiation through to completion of senescence. Gene expression certainly changed over each experimental period, but there was no consistent relationship to stage of senescence and nothing to suggest that a particular set of genes represented a senescence programme. This leads to the conclusion that transcriptional patterns during tree leaf senescence represent a timetable (Keskitalo *et al.*, 2005) rather than a programme. Inferring the existence and functions of genetic programmes, networks and circuits from 'omics data is at the heart of the SB philosophy. Perhaps particularly in the case of plants, it is important to bear in mind that the urge to keep uncontrollable environmental influences out of the picture has its dangers.

An implication of this conclusion concerns the current Minimum Information About a Microarray Experiment (MIAME) checklist, which researchers are required to use when submitting transcriptomics data to public databases. As currently implemented, MIAME cannot easily accommodate descriptions of field-based experiments. Discussion led to the conclusion that MIAME and other minimum-information checklist standards will need expansion to include the whole gamut of research on plant-based systems.

Can SB approaches aid ecological and agricultural studies (and vice versa)?

Molecular ecology considers the fitness of particular genes in particular environments. The flow of transgenes between crops and their wild relatives is a test-bed for the study of how the fitness game is played out in the natural world. Mike Wilkinson (Aberystwyth University, UK) discussed this subject from the systems perspective. Using the example of cultivated and wild *Brassica* species in the UK (Ford *et al.*, 2006), he showed that it is necessary to range across extremes of scale to gather the data necessary to model and predict. These studies extend from the level of the genes themselves, defined in molecular (DNA sequences) and cytogenetic (genomic regions) terms, all the way up to determination of landscape-level distributions of wild and cultivated populations by terrestrial ecological analysis and remote sensing. The objective is to construct a predictive model of gene flow and

its ecological consequences that characterizes the selection pressures experienced by the wild recipients and their community associates on introduction of a novel gene.

Andy Taylor (Swedish University of Agricultural Sciences, Uppsala, Sweden) similarly defined a system that transcends scale and highlights the opportunities and challenges of driving the SB approach out from the cell and into the big wide, often hostile world. The ectomycorrhizal (ECM) symbiosis in boreal ecosystems is characterized by species richness and taxonomic diversity amongst the soil fungi that associate with the roots of a range of woody perennials (Toljander *et al.*, 2006). Taylor argued that there are many parallels between the approaches, concepts and ideas of SB and those used within ecosystems ecology. He showed that redundancy and modularity are principles underlying biological robustness in ecosystems as they do in narrow-sense SB. System degradation (both graceful and catastrophic) is also a feature of behaviour at both scales. Furthermore, system perturbation is a powerful tool for probing functions and interactions; in the case of the nutrient-poor boreal ecosystem, addition of nitrogen elicits a dramatic response from ECM fungal communities and such interventions are proving useful in developing and validating mechanistic models. It is undeniable that, at such extremities of scale and biological complexity, SB and agro-ecological systems biology stand either side of a yawning chasm. However, the present Symposium established that there is already a potential for a convergence of thinking and tools that can bridge the gap.

What are the grand challenges in plant biology that SB can help address?

The 21st century will be dominated by three global grand challenges: climate, energy and food. These are clearly linked both in origin and in the potential remedies for the problems they represent. Fundamental knowledge and practical applications of plant science are at the heart of humanity's response to the testing times it faces. The Symposium agreed that systems biologists and biologists of systems need to make common cause if taxpayers, charities and businesses are to continue to invest in the promises made by the research community. As William Blake put it: 'I must create a system, or be enslaved by another man's.' Whether the goal is the C₄ rice plant, the sustainable mycorrhiza-based forest ecosystem, the *in silico* Arabidopsis or the perfect rose, it seems clear that the future of plant science has to lie in engagement with biological systems in their entirety.

Howard Thomas

Institute of Biological Science, Edward Llwyd Building,
Aberystwyth University, Ceredigion SY23 3DA, UK
(tel +44 1970 628768; fax +44 1970 622350;
email hot@aber.ac.uk)

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